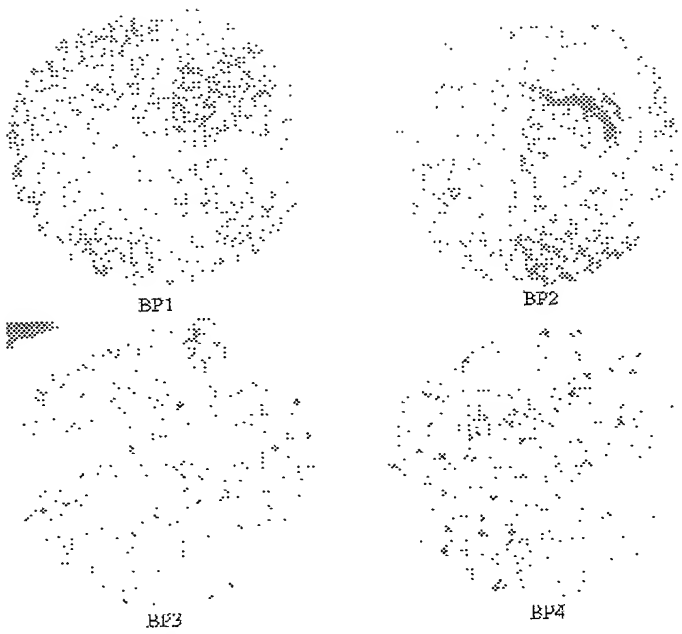


Figure 1 (A-D in row order): Identification of a phage displaying peptide sequence of Sirt2 by plaque lift: E.coli BLT5615 infected with amplified phage library after biopanning 1-4 were plated onto LB-Agar plates and plaque lifts were performed for all the individual plates. The plaque lift filter membranes were then hybridized with a Sirt2 cDNA probe. The percentage of positive plaques (number of positive plaques/total number of plaques x 100) as determined for each plates labeled BP1-4, figure 1 increased with each successive cycle of biopanning. For BP1and BP2 the percentage of positive plaques was negligible. For BP3 and BP4, percentage of positive plaques was 1.7 % and 8.6 % respectively.

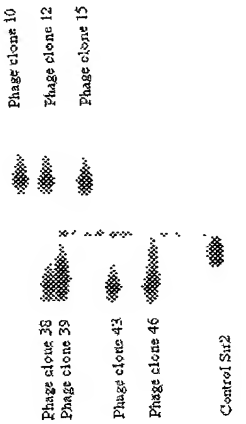


Figure 2: Analysis of the PCR product of the plaques by Southern Blot hybridization: In order to confirm that those positive plaques contain phage clones

displaying the peptide sequence of Sirt2, we randomly picked up 50 plaques and PCR amplified each insert using T7 coat protein forward primer (5′ TCTTCGCCCAGAAGCTGCAG 3′) and T7 coat protein reverse primer (5′ CCTCCTTTCAGCAAAAAACCCC 3′). Filter hybridization was performed using the same Sirt2 cDNA probe as above. As shown in the figure 2, 7 out of 50 plaques (14%) hybridized to the Sirt2 probe, a frequency similar to that observed in the plaque lifts. Plaques positively reacting with the *Sirt2* probe were picked and also hybridized on Southern Blots of PCR product, (data not shown).

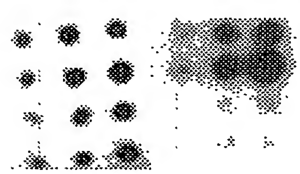


Figure 3
A= 10^7 pfu;
B= 10^8 pfu;
C= 5 X 10^8 pfu

***Figure 3: Dot Blot analysis of Sirt2 positive plaques (figure 3)*:** We chose Sirt2 positive plaques (upper two rows) and Sirt2-negative plaques (lower two rows) and 1 μl (pfu indicated at left) of each amplified phage clone was spotted onto the nitrocellulose membranes which were then treated as if they were standard immunoblots using our rabbit polyclonal Sirt2 antibody (right panel) or a mouse monoclonal antibody to the T7 capsid protein (left panel). The Sirt2 antibody reacted specifically with the Sirt2 phage. The identity of the phage was confirmed by direct PCR sequence analysis of the cDNA inserts in two independent Sirt2 positive phage. Thus we can isolate phage expressing the epitope to which our antiserum was directed and can distinguish it from other phage.

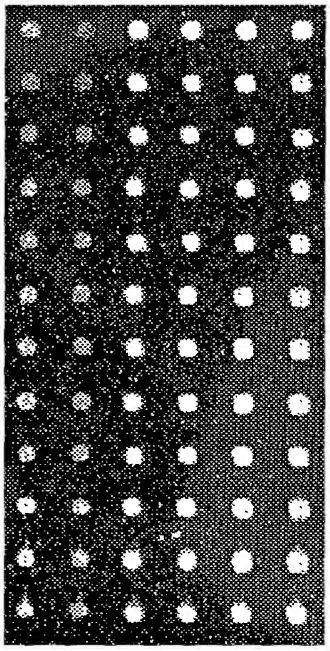


Figure 4: Immunological Detection of Phagotopes on Microarrays: We spotted microarrays using Sirt2 T7 clones and other T7 clones that do not express Sirt2. We used these arrays to analyze a mixture of Cy5-labeled (red) rabbit Sirt2-immunized serum and Cy3-labeled (green) T7 coat protein antibody (Novagen) added to the pre-immune rabbit serum. The scanned two-color image clearly shows specific detection of the Sirt2-expressing T7 clones by the anti-Sirt2 antibody. The Sirt2 expressing clones appear yellow since they bind both the red-labeled anti-Sirt2 antibody and the green-labeled anti-T7 antibody. The non-Sirt2-expressing T7 clone are green as they only bind to the Cy3-labeled anti-T7 antibody. This development of detection of protein epitopes in bacteriophage bodes well for the applicability of phage arrays to the detection of low abundance species and weak binders. The spots in the image are approximately 100 microns in diameter.

Labeled Red:
Sirt2-immunized rabbit serum
detected using Cy5-conjugated
anti-rabbit IgG

Labeled Green:
Anti-T7 antibody Cy3-conjugated

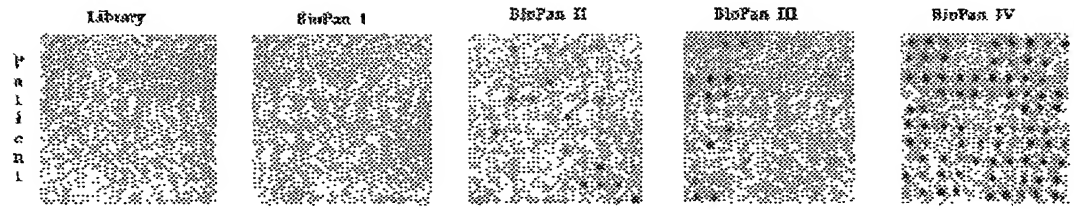
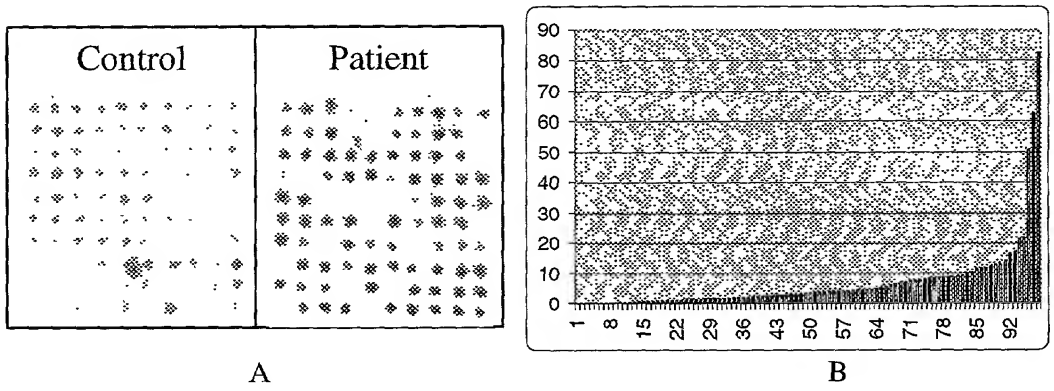


Figure 5: ECL detection of phagotopes selected with a breast cancer patient's serum: Library and BP1-4



A B

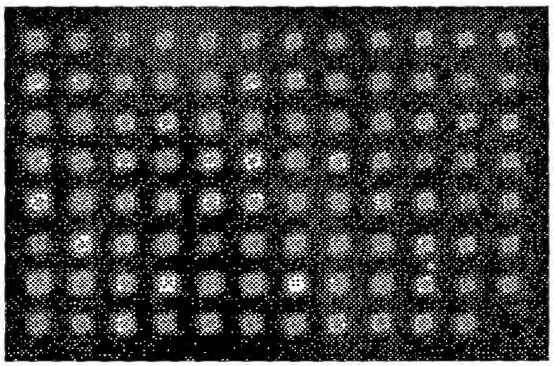


Figure 6: A. Comparison of serum reaction of control and breast cancer patient with phagotopes from BP4. B. The BP4 filters were scanned and the ratio of the pixel densities plotted in rank order. C. Clones from Patient #1 biopanning reacting with antibodies in the same serum sample on a phagotope microarray. The antibody reaction is detected with a Cy5-labeled antibody to human IgG and the control Cy3-labeled antibody to phage T7 capsid protein. Green spots indicate no reaction with human IgG and yellow spots indicate an reaction with both antibodies.

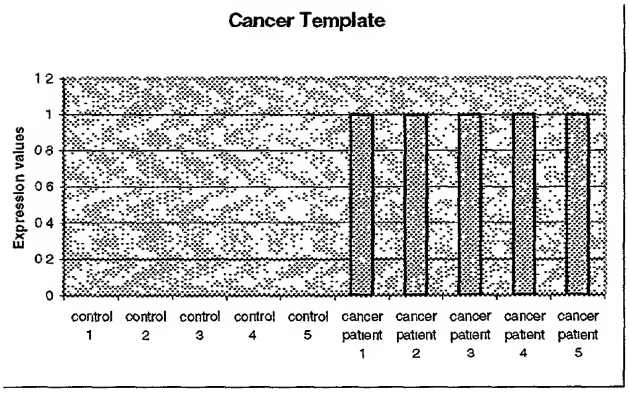


Figure 6. Clone template definition. The cancer template will look for epitopes reactive in cancer and non-reactive for healthy subjects.

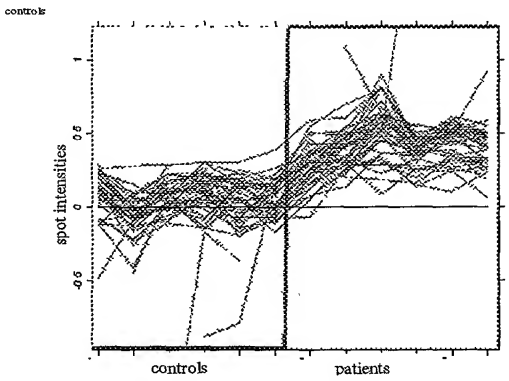


Figure 7. Finding informative epitopes: The spot intensities are plotted on the vertical axis for 12 subjects (controls to the left and patients to the right). We used the template defined on the left (shown in blue) and a correlation distance. A correlation threshold of 0.8 selected the 46 epitopes shown here in red (out of the total of 4x96=384 shown here in yellow).

5

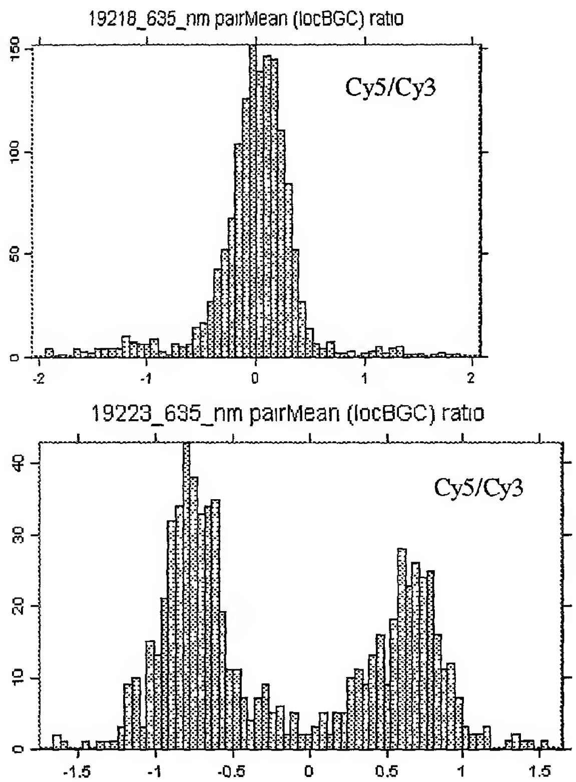


Fig. 8. An example comparison between the histogram of a control subject (19218) with a high but non-specific reaction to the left, and the histogram of a patient (19223), to the right. The histograms are calculated on the ratios of the background corrected mean intensity of the human IgG labeled with Cy5 vs. the background corrected mean intensity of the T7 labeled with Cy3.

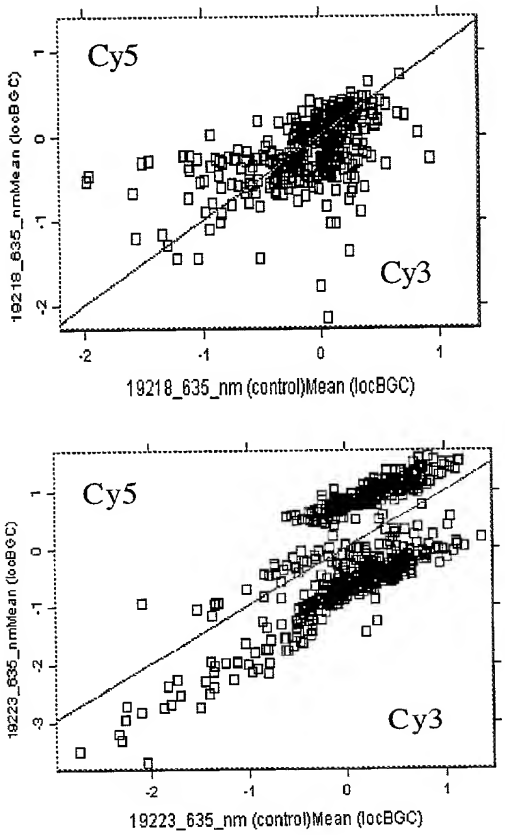


Fig. 9. A comparison between the scatterplot of a control subject (19218) with a strong but non-specific reaction and the scatterplot of a patient MEC1 (19223). The scattergrams plot the background corrected mean intensity of the human IgG labeled with Cy5 vs. the background corrected mean intensity of the T7 labeled with Cy3.

Clones from patient	Source of sera 1	2	3	4	5	6	7	8	9	10	11	12
1	1											
2		1										
3			1									
4				1								
5					1							
6						1						
7							1					
8								1				
9									1			
10										1		
11											?	
12												?

Fig. 10. The matrix of reactivity between sets of clones coming from patients 1-12 (in rows) and sera from same patients (in columns). At this point (step 2 of Procedure 2), the matrix contains the results of the self-reactions: patients 1-10 have a specific self-reaction whereas

patients 11 and 12 do not. Patients 11 and 12 are eliminated from the clone selection procedure.

Clones from					Source of sera					
patient	1	2	3	4	5	6	7	8	9	10
2		1		1				1		
3			1			1				1
5					1			1	1	
7							1			1
8								1		
9									1	
10										1
1	1									
4				1						
6						1				

Fig. 11. Matrix of reactivity between sources of clones and different sera ordered by reactivity. The clones from patient 2 react with sera from self (column 2) and patients 4 and 8. The clones from patient 3 react with sera from self (column 3) and patients 6 and 10, etc. Note that the union of the set of clones coming from patients 2, 3, 5, 7 and 1 will ensure that the chip made with these clones reacts with all patients.